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AID for Innate Immunity to Retroviral Transformation

AID is a cytidine deaminase essential for class switch recombination and somatic hypermutation during the humoral immune response. In this issue of *Immunity*, Gourzi et al. (2006) show that AID also plays a critical role in innate immunity to virally induced acute pro-B cell leukemia.

For more than a generation, immunologists had been captivated (some might say distracted) by the elegance of adaptive immunity. Progress in understanding combinatorial diversity of antigen receptors, clonal selection, immune memory, and self-tolerance contributed to a view that innate immunity was but a modestly effective stop-gap measure until the antigen-specific lymphocytes arrived to save the day. The argument was made that given the rapid rate of microbial evolution, innate immunity was forever destined to be out-gunned. Recently, however, we've been dazzled by the discovery of a growing array of innate defense mechanisms that recognize and attack microbes at levels inaccessible to the adaptive immune system, and we have achieved a deeper appreciation of the myriad of intricate interactions between the two. Interferons, for example, activate innate viral defense mechanisms and enhance responses of T cells specific for virally infected cells. Recently, a family of enzymes known as cytidine deaminases, originally discovered due to their ability to edit cellular RNA molecules, was found to play a key role in innate immunity to virus infection as well as more globally in the adaptive humoral immune response (Turelli and Trono, 2005). And now, as reported in this issue of *Immunity* by Gourzi et al. (2006), one member of this family appears to provide a first line of defense against a cancer-causing retrovirus (Figure 1).

The initial discovery of a role for cytidine deaminases in antiviral immunity came from ground-breaking studies by Malim and colleagues on HIV (Sheehy et al., 2002). The HIV genome encodes a protein, vif (viral infectivity factor), which is essential for its ability to infect most target cells. Sheehy et al. used subtractive cDNA cloning to identify the missing gene in a mutant T cell line which could be infected by a vif-deficient virus. This gene, initially named *CEM15*, was found to encode APOBEC3G, a paralog of the cytidine deaminase APOBEC1, an RNA editing enzyme. Subsequent work revealed that vif targeted APOBEC3G for ubiquitin-dependent degradation, preventing its inclusion in virions (Sheehy et al., 2003). The idea was that APOBEC3G, through its deaminase activity, would inactivate virus by introducing a large number of C-to-U modifications in the minus strand of viral cDNA. These modifications would either destroy the reverse transcript or introduce debilitating numbers of point mutations. HIV had evolved a way to evade this newly appreciated innate defense mechanism and perhaps even use it to enhance the rate of its own evolution. In short order, several groups replicated these results and extended them to show the involvement of APOBEC3G in the cellular response to retroviruses in general and possibly other classes of RNA viruses such as HBV (Turelli and Trono, 2005).

This was not the first surprising example of the involvement of cytidine deaminases in immunity. Several years earlier, Honjo and colleagues discovered that another APOBEC family member, activation-induced cytidine deaminase, or AID, was necessary for both somatic hypermutation (SHM) and class switch recombination (CSR) (Muramatsu et al., 2000). They found *AID* among a set of genes activated when B cells are treated with cytokines and LPS to induce CSR in vitro. Remarkably, the *AID* gene mapped to the site of an inherited human immunodeficiency disease, HIGM2, and was mutated in patients with the disease (Revy et al., 2000). These patients lack immunoglobulin (Ig) isotypes other than IgM and IgD and fail to undergo affinity maturation by SHM during an immune response. A targeted mutation in the AID gene duplicates the human disorder in experimental mice. Although there is still some debate in the field, the predominant view is that AID activates both CSR and SHM by the direct deamination of cytosine residues in genomic DNA, leading to DNA breaks and either recombination or error prone repair. It is this ability of AID to cause genomic DNA damage that underlies the remarkable observations made in the current report from Papavasiliou and colleagues (Gourzi et al., 2006).

Gourzi et al. ask whether AID, like other APOBEC family members, might play a role in the host response to viral infection. Abelson murine leukemia virus (A-MuLV) is a defective retrovirus that does not replicate in infected cells. Instead, it transforms murine pro-B cells in vitro and causes acute pro-B cell leukemia in vivo by transducing the *v-Abl* oncogene (Rosenberg, 1982). Gourzi et al. found that AID-deficient bone marrow (BM) cells infected with A-MuLV were more susceptible to transformation, resulting in increased number and size of transformed foci as compared to wild-type BM cells in vitro. Because death of infected wild-type and AID-deficient BM cells was identical, the authors conclude that AID functions to restrict proliferation of infected cells. Animal experiments revealed far more rapid death due to fulminate leukemia of mice injected with infected AID-deficient BM cells as compared to mice infected with wild-type BM cells. Gourzi et al. went on to show that A-MuLV induces *AID* transcription in infected pro-B cells but to an amount lower than that found in mature germinal center B cells undergoing CSR. Unlike APOBEC3G, AID does not deaminate the viral genome but instead activates somatic mutation in infected cells as demonstrated by the appearance of mutations in both *Ig* and *λ5* loci. The authors propose that host genome damage caused by AID expression is key to its protective effect. They showed that infected cells activate a Chk1-dependent genotoxic stress

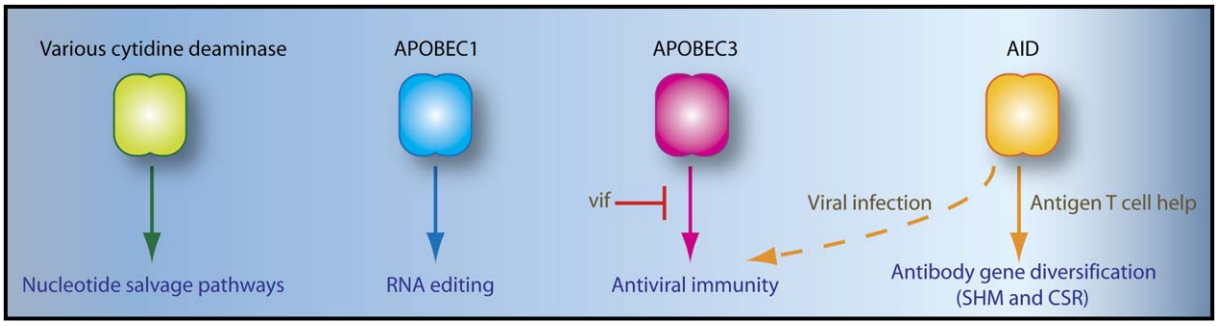

Figure 1. Cytidine Deaminase Activities in Animal Cells

Cytidine deaminases (CDAs), long known to be involved in nucleotide salvage pathways, have more recently been found to play key roles in gene expression, anti-viral immunity, and immunoglobulin gene diversification (SHM, somatic hypermutation; CSR, class-switch recombination). Vif is an HIV gene product which interferes with APOBEC3 activity.

pathway observed in other transformed cells, resulting in cell cycle arrest and upregulation of the NK cell activating ligand Rae-1 (Gasser et al., 2005). Thus, AID activation would slow infected cell growth and make the cells targets for NK cell killing.

Although this new study uncovers an unexpected role for AID in innate immunity, it is at first glance hard to reconcile with our current understanding of the function of AID in germinal center B cells where it is abundantly expressed. Why doesn't AID expression cause genotoxic stress in germinal center B cells, restricting B cell proliferation and increasing their susceptibility to NK cell lysis? According to the authors, Rae-1 is indeed induced in germinal center B cells. One possibility is that cell cycle arrest due to the action of p53 may be prevented by the germinal center B cell transcription factor Bcl-6, which was recently shown to interfere with p53 activation (Phan and Dalla-Favera, 2004). Because Bcl-6 is not expressed in pro-B cells, AID induction should more readily lead to cell cycle arrest and apoptosis in A-MuLV-infected pro-B cells. It is worth noting in this regard that about 50% of *Abl*-transformed pro-B cell lines contain *p53* mutations (Unnikrishnan et al., 1999). In regards to NK cell lysis, it may be relevant that susceptibility to NK killing depends on the ratio of inhibitory and activating ligands on target cells. Transformed cells often show diminished expression of MHC-I molecules, the main inhibitory ligand for NK cells. Perhaps germinal center B cells express sufficient MHC-I to prevent NK cell killing regardless of Rae-1 induction, whereas A-MuLV-infected cells might lose MHC-I expression favoring NK cell killing.

The in vivo data in Gourzi et al. represent compelling evidence that AID serves a protective role against A-MuLV-induced pro-B cell leukemia. However, it is still not clear whether this protection is mediated by NK cell activation, Chk1 induced cell cycle arrest or some other unsuspected mechanism. As the authors point out, animal experiments involving either NK cell depletion or Rae-1 antibodies to block ligand-receptor interactions will be required to confirm the role of NK cells in A-MuLV immunity. Additionally, it will be interesting to determine whether AID or other APOBEC family members play a role in host defense against different cancer-causing viruses. Alternatively, as suggested by others, genomic instability is a common feature of transformed cells, which may lead to NK cell activating ligand expression independent of APOBEC expression (Gasser et al., 2005). Finally, these results pose the interesting evolutionary question of whether two of the most elegant mechanisms of adaptive immunity, CSR and SHM, actually evolved from a component of innate immunity.

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Selected Reading

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